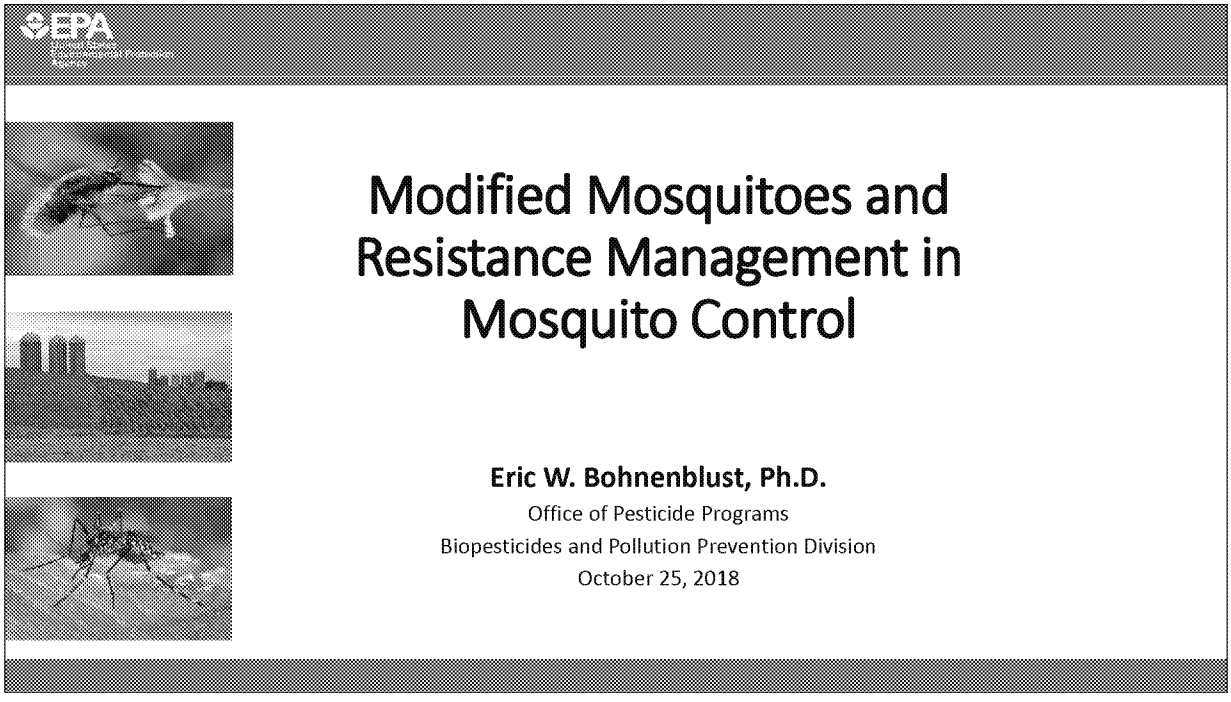
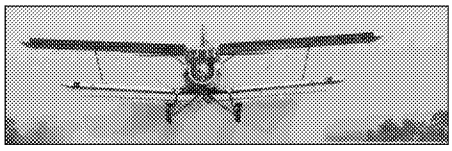


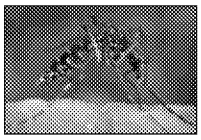
Current Approaches to the Environmental Risk Assessment of GE Crops

June 27-29, 2011

Introduction

- Mosquito Adulticides + Larvicides
  - Active ingredients (a.i.), how they're used
  - Mosquito management
  - Resistance management (RM)
- Modified Mosquitoes
  - Regulatory jurisdiction
  - Regulatory considerations
  - Species
  - Sterile insect techniques
- Wolbachia Mosquitoes
  - What are they?
  - How do they work?
- GM Mosquitoes
  - What are they?
  - How do they work?





Mosquito Adulticides and Other Tools for Adults

Registered a.i.s for wide-area application: malathion, naled, permethrin, pyrethrins, etofenprox, chlorpyrifos, d-phenothrin, prallethrin, sumithrin, deltamethrin

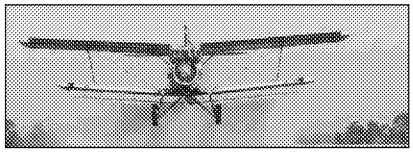
- Organophosphates and pyrethroids
  - Chemicals with a broad spectrum of activity against pests
- Ultra low volume (ULV), truck mounted, or aerial applications
  - Spray directly contacts mosquito
  - No residual activity
  - www.epa.gov/mosquitocontrol/controlling-adult-mosquitoes

Registered a.i.s for residual adulticide applications: acetamiprid, bifenthrin, permethrin, zeta-cypermethrin

- Neonicotinoids and pyrethroids
- Typically applications to surfaces
  - mosquitoes must land on treated surface

Other tools

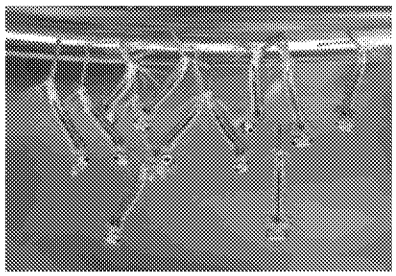
- Insecticide treated clothing, repellents, consumer products
- Pyriproxyfen/*Beauveria bassiana* trap with ovicidal/adulticidal properties



Larvicides for Mosquito Control

Registered ingredients for mosquito larviciding: spinosad, *Bacillus thuringiensis israelensis*, methoprene, *Bacillus sphaericus*, pyriproxyfen, mineral oil, temephos

- Typically applied as tablets or granules
  - Oil is a surface film that suffocates larvae
- Can be applied by hand or aerially
- Often different modes of action than adulticides



www.epa.gov/mosquitocontrol/controlling-mosquitoes-larval-stage

Pesticide Resistance

Resistance - a heritable change in the sensitivity of a pest population

- Observed by repeated failure of a pesticide to achieve the expected level of control when used according to label recommendations for that pest species. (IRAC - www.irac-online.org/about/resistance/)

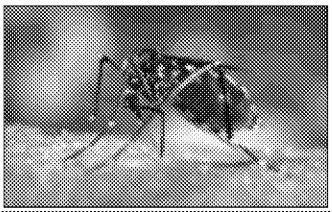
Cross-resistance - when resistance to one insecticide confers resistance to another insecticide, even where the insect has not been exposed to the latter product. (IRAC - www.irac-online.org/about/resistance/)

- Often occurs within a chemical class (e.g., pyrethroids)

Mosquitoes resistant to a single class of insecticide reported in 61 countries, including USA

- resistance to 2 or more classes reported in 50 countries

(www.who.int/malaria/areas/vector_control/insecticide_resistance/en/)



Consequences of Pesticide Resistance

Resistance reduces the utility and lifespan of pesticides

- commercial use
  - Permethrin resistance reduces the effectiveness of permethrin as an adulticide
- consumer use products
  - Pyrethroid resistance in mosquitoes can reduce effectiveness of personal/military use of treated clothing articles (Agramonte et al. 2017)

Pesticide Treadmill

- When a pesticide fails because of resistance, scientists must develop new pesticides or applicators rely on another pesticide class which in turn fails, and scientists create another pesticide and so on (Scott, D. 2005)

Resistance management prolongs the usefulness of pesticides

Agramonte NM, Bloomquist JR, Bernier UR (2017) Pyrethroid resistance alters the bloodfeeding behavior in Puerto Rican Aedes aegypti mosquitoes exposed to treated fabric. PLoS Negl Trop Dis 11(9): e0005954. https://doi.org/10.1371/journal.pntd.0005954
Scott, D. 2005. The magic bullet criticism of agricultural biotechnology. https://link.springer.com/content/pdf/10.1007/s10806-005-0632-4.pdf

Managing Mosquito Populations – Resistance Management

EPA has no resistance management labeling requirements for mosquito adulticides

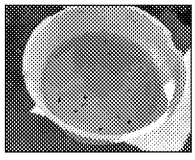
EPA website provides guidance on controlling mosquitoes through an integrated approach
www.epa.gov/mosquitocontrol/success-mosquito-control-integrated-approach
www.epa.gov/mosquitocontrol/joint-statement-mosquito-control-united-states
includes:

- Removing mosquito larval habitats (e.g., buckets, bird baths, fountains)
- Use of structural barriers (e.g., windows, screens)
- Larviciding (e.g., Bti, insect growth regulators)

Alternative approaches for control are important for managing resistance to adulticides

Monitoring resistance is also important for managing mosquitoes & resistance

- Resistance usually determined using standard WHO susceptibility assays (bottle assay)
  www.who.int/malaria/publications/atoz/9789241511575/en/



Additional Resistance Management Strategies

Rotate chemical class of products applied to avoid consecutive applications of products in the same chemical class

Monitor mosquito populations, spray when target species are present or populations reach monitoring thresholds

- Avoid applications when target species is not present

Time application to times and locations of peak activity to contact the most individuals
(e.g., mornings and evenings for crepuscular mosquito species)

Outreach and education

- Public can alert local control districts to problem areas
- Teach public to remove mosquito habitats, reducing populations

Guidance for Labeling to Address Resistance Management

Pesticide Registration Notice 2001-5 - www.ceris.purdue.edu/info/prnotice/pr2001-5.pdf

- Mode of action symbols and other RM statements

Pesticide Registration Notice 2017-1 - www.epa.gov/sites/production/files/2017-09/documents/prn-2017-1-pesticide-resistance-management-labeling.pdf

- Revision and update of PRN 2001-5

These guidance documents provide voluntary resistance management language

- Intended for agricultural products
- Do not apply to products for consumer use
- Typically does not apply to wide-area mosquito adulticide products
- **NOT MANDATORY**

Example Resistance Management Language on Label

NOVALURON GROUP 15 INSECTICIDE

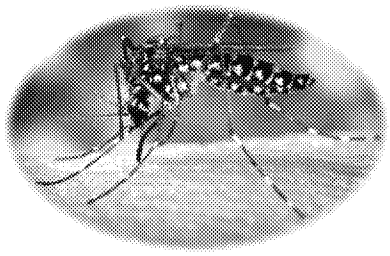
[Insecticide for use on Avocado*, Beans, Berries (Low-Growing), Bushberries, Carrot*, Cotton, Cucurbit Vegetables, Fruiting Vegetables, Head and Stem *Brassica*, Leafy *Brassica* Greens*, Ornamentals (Container Grown Ornamentals in Greenhouses, Shadehouses, Outdoor Nurseries), Peanuts*, Pears*, Pome Fruits, Potatoes / Sweet Potatoes, Sorghum*, Soybeans*, Stonefruits, Strawberry, Sweet Corn, Sugarcane*, Swiss Chard*, and Turnip Greens*.
*Not Registered For Use in California]

Active ingredient:	(% by weight)
Novaluron (1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea)*	9.3%
Other ingredients:	90.7%
Total	100.0%

*Contains 0.83 lbs. of novaluron per gallon.

What Are Modified Mosquitoes?

- Mosquito-related products intended to function as pesticides for mosquito population control purposes, and that are not intended to cure, mitigate, treat or prevent a disease
- Genetic material incorporated into mosquitoes
- Microbial pesticides (*Wolbachia*) stably incorporated in mosquitoes



Regulatory Jurisdiction of Modified Mosquitoes

- **FDA guidance 236** – indicates which "mosquito" products are regulated by FDA vs. EPA
  - www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM533600.pdf

Examples of Products Regulated by FDA (New Animals Drugs)

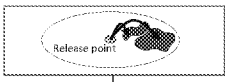
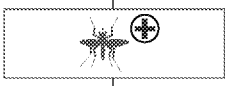
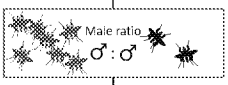
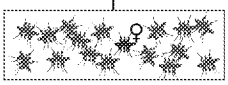
- Products intended to reduce the virus/pathogen load within a mosquito, including reduction in virus/pathogen replication and spread within the mosquito and/or reduction in virus/pathogen transmissibility from mosquitoes to humans.
- Products intended to prevent mosquito-borne disease in humans or animals.

Products Regulated by EPA (Pesticides)

- Products intended to reduce the population of mosquitoes (for example, by killing them at some point in their life cycle, or by interfering with their reproduction or development).*

*This is true whether the product is a traditional chemical product or involves a different technology (e.g., a recombinant DNA construct or bacteria intended to reduce the population of mosquitoes).

Special Considerations for Mosquitoes

Unique Consideration		Data or Analysis
Dissemination is mosquito dependent		• Release point ≠ treatment area • Definition of application rate
Health of released mosquitoes		• Age of mosquitoes at time of release • Handling conditions during shipping • Fitness cost of new trait
Density of existing wild mosquito population		• Establish release numbers according to population density
Accidental female release		• Likelihood? • ID mosquito species (species-specific) • Potential human health and eco implications

Registered and Pending and Future "Modified" Mosquito Products

Current Products

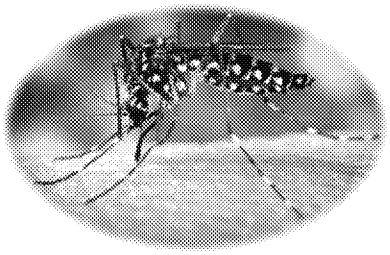
- *Wolbachia*-infected *Aedes albopictus* mosquitoes – EPA Reg. No. 89668-4
- *Wolbachia*-infected *Aedes aegypti* mosquitoes – EPA Reg. No. 89668-EUP-3 (experimental use permit)

Pending Actions

- OX513A genetically modified *Ae. aegypti* mosquitoes (EUP application)

What might we see in the Future?

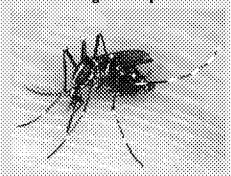
- Gene editing (e.g., CRISPR), Gene drives



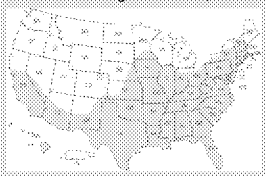
Targeted Mosquito Species

***Aedes albopictus* (Asian tiger mosquito)**

Asian tiger mosquito



Estimated range in the U.S., 2016



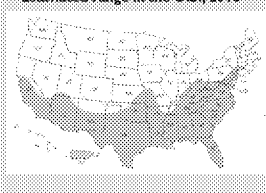
- Prefer forested areas
- Active during the day
- Can carry Zika and other viruses of concern for human health
- Invasive to U.S.

Source: CDC

***Aedes aegypti* (Yellow fever mosquito)**

Yellow fever mosquito

Estimated range in the U.S., 2016



- Anthropophilic
- Active during the night
- Can carry Zika and other viruses of concern for human health
- Invasive to U.S.

Source: CDC

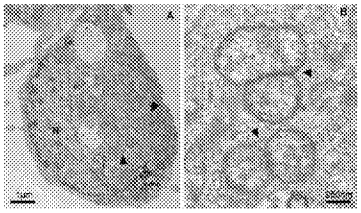
Sterile Insect Technique – Theory Behind Modified Mosquitoes

- Sterile Insect technique (SIT)
  - Introduction of sterile insects and thus sterility into wild populations to achieve population suppression/control
- Typically, released sterile males to mate with wild-type females
  - Sterilized by radiation
  - USDA program to control screwworm started in 1950's
    - https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/cattle-disease-information/nws/new-world-screwworm
  - Also used extensively for fruit fly control
- Usually part of an Integrated Pest Management (IPM) program
- Species specific control tactic
  - Control is not immediate, but may take 1-2 generations to achieve
  - Releases must continue for the entire season/year

Dyck, VA. et al. Eds. 2005. Sterile Insect Technique: Principles and Practice in Area-Wide Pest Management.

Wolbachia Bacteria

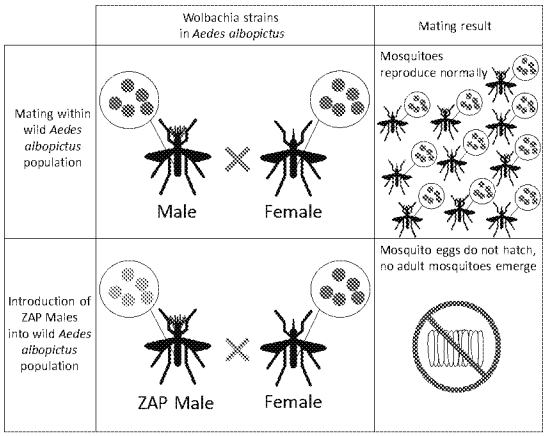
- *Wolbachia pipientis* is a common obligate intracellular bacterium found in an estimated 65% of insect species (Hilgenboecker et al. 2008)
  - 1 million named and estimated 5.5 million insect species (Stork 2018)
- *Wolbachia* infection identified in wild *Culex* spp. mosquitoes in 1971 (Yen and Barr 1971)
- Cytoplasmic incompatibility resulting from *Wolbachia* infection discovered early to mid-20th century (Hertig and Wolbach 1924; Laven 1951)
- Vertically transmitted from mother to offspring (Werren et al. 2008)



McMeniman et al. 2008

- Hertig, M. and S.B. Wolbach. 1924. Studies on rickettsia-like micro-organisms in insects. The Journal of Medical Research. 44: 329.
- Hilgenboecker, K., P. Hammerstein, P. Schlattmann, A. Telschow and J.H. Werren. 2008. How many species are infected with *Wolbachia*?–a statistical analysis of current data. FEMS Microbiology Letters. 281: 215-220.
- Laven, H. 1951. Crossing experiments with *Culex* strains. Evolution. Vol. 5: 370-375.
- McMeniman, C.J., A.M. Lane, A.W. Fong, D.A. Voronin, I. Iturbe-Ormaetxe, R. Yamada, et al. 2008. Host adaptation of a *Wolbachia* strain after long-term serial passage in mosquito cell lines. Applied and Environmental Microbiology. 74: 6963-6969.
- Werren, J.H., L. Baldo and M.E. Clark. 2008. *Wolbachia:* master manipulators of invertebrate biology. Nature Reviews Microbiology. 6: 741-751.
- Yen, J.H. and A.R. Barr. 1971. New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens* L. Nature. 232: 657.

Wolbachia and Cytoplasmic Incompatibility



Female \ Male	U	A	B	AB
U	*U*	–	–	–
A	*A*	*A*	–	–
B	*B*	–	*B*	–
AB	*AB*	*AB*	*AB*	*AB*

Fig. 1. Diagram of possible crossing compatibility between uninfected (U), single-infected (A or B) and superinfected (AB) lines. Dashes indicate cytoplasmically incompatible crosses in which egg hatch is reduced or eliminated. Letters within cells indicate the infection type of the resulting brood assuming maternal inheritance.

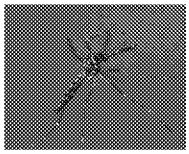
Dobson et al. 2001

- ZAP Males are not sterile, but they cannot reproduce with females of wild populations
- During cell division, DNA is not distributed correctly to offspring – not viable

Dobson, S.L., E.J. Marsland and W. Rattanadechakul. 2001. Wolbachia-induced cytoplasmic incompatibility in single-and superinfected *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology. 38: 382-387.

Wolbachia Infected Mosquitoes

Wolbachia strain used in product does not occur in wild *Aedes* mosquitoes



Aedes spp. infected with a *Wolbachia* found naturally in other insect species.

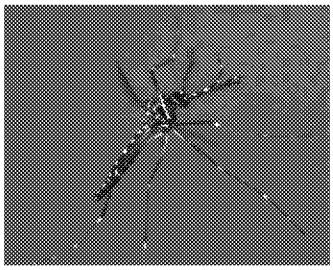
EPA Reg. No. 89668-4 registered in 2017 for use in: California, Connecticut, Delaware, Illinois, Indiana, Kentucky, Massachusetts, Maine, Maryland, Missouri, New Hampshire, New Jersey, Nevada, New York, Ohio, Pennsylvania, Rhode Island, Tennessee, Vermont, the District of Columbia, and West Virginia

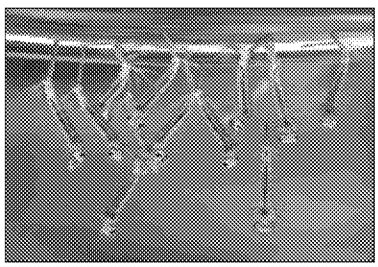
EPA Reg. No. 89668-EUP-3 experimental permit granted in 2015 for testing in Florida, California, and Texas

https://www.regulations.gov/docket?D=EPA-HQ-OPP-2016-0205
https://www.regulations.gov/docket?D=EPA-HQ-OPP-2017-0392

Current EUP Action for OX513A

- Intended for field trials against *Ae. aegypti* in Monroe County, Florida and Harris County, Texas
- Requesting EUP for 2 years to assess efficacy and dispersal in US
- Notice of receipt published April 9, 2018
  - Received approximately 232,000 public comments



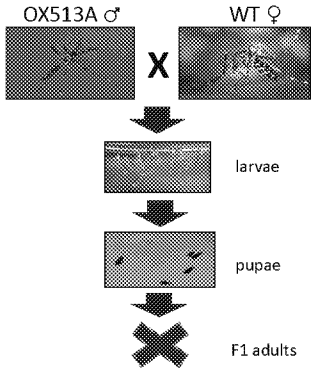


OX513A *Aedes aegypti* Mosquitoes

- Released sterile adult male OX513A mosquitoes are homozygous for an rDNA construct (tTAV)
- tTAV construct is passed to F1 offspring resulting from male OX513A X wild type female matings
  - In absence of tetracycline tTAV results in lethality to progeny prior to adult emergence
  - When reared in presence of tetracycline, larvae survive to adulthood

Release of Insects Carrying a Dominant Lethal Gene

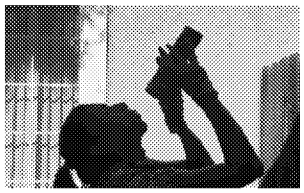
- OX513A males mate with wild females
- Offspring die before reaching adulthood



www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVM/AnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/UCM514695.pdf
Wise de Valdez. M., et al. 2011. Genetic elimination of dengue vector mosquitoes. Proc. Natl. Acad. Sci. https://doi.org/10.1073/pnas.1019295108

Commonalities Between *Wolbachia*-Infected Mosquitoes and Oxitec Product

source: www.oxitec.com

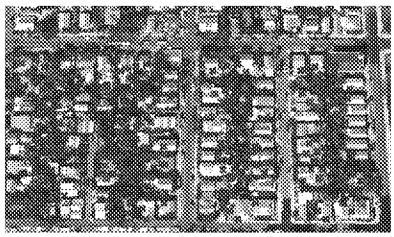


source: www.mosquitomate.com

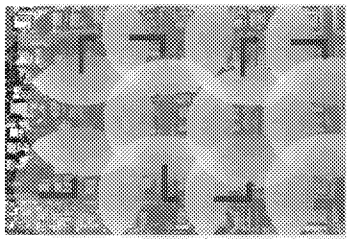
- Release of live adult male mosquitoes into the environment
- Offspring of these males cannot develop into adulthood
- Species-specific effect
- Reduces mosquito population by hindering successful reproduction

Only female mosquitoes bite

Individual larvicide placement



Mosquito-related biopesticide release points

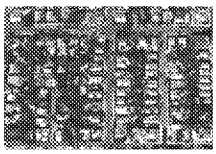


Source: Oxitec

Why are Modified Mosquitoes Important?

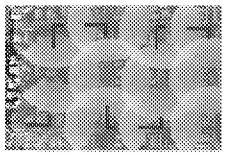
- *Ae. albopictus* & *Ae. aegypti* can vector diseases such as: dengue, Zika, equine encephalitis, and West Nile
- Alternative to mosquitocides for which resistance has been reported
- Highly specific tool to locally reduce specific *Aedes* spp. mosquito populations
- Can be used in conjunction with IVM programs such as larvicides and adulticides (if appropriately timed)
- Reaches areas that adulticide sprays can't reach and those areas too small to be treated with larvicides

Individual larvicide placement



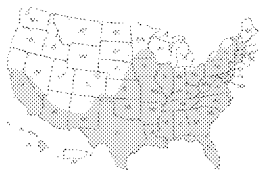
VS.

Mosquito release points

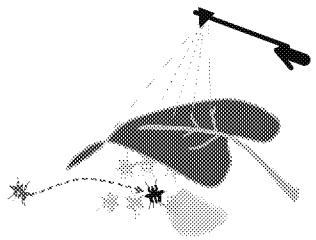


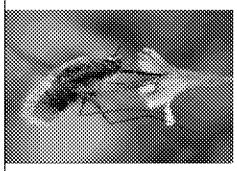
Source: www.oxitec.com

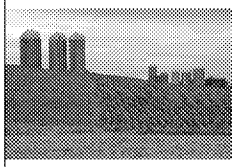
Estimated range of *Aedes albopictus* in the U.S., 2016

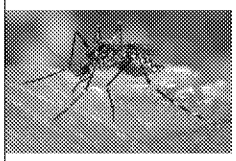


Source: CDC









Questions?